



First record of a cyanobacterium *Petalonema alatum* (Borzi ex Bornet & Flahault) Correns (Cyanobacteria, Scytonemataceae) in Africa

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Abstract

Petalonema alatum (Borzi ex Bornet & Flahault) Correns is a nitrogen fixing, subaerial cyanobacterium characterized by a blue-green trichome surrounded by a very broad, lamellated mucous sheath. It typically grows on dripping limestone rocks in temperate regions, but it has also been observed in some calcareous lakes and limestone springs. Although the species is known to be present in the Americas, Europe and Asia, no records could be found for its presence in Africa. In the last decade, it was sampled twice from rock surfaces in cave overhangs in the Free State Province of South Africa, representing a first record of its presence in Africa. A taxonomic description, microscope images as well as detailed geographical distributions of *P. alatum* are provided.

Key words

Algae; caves; cyanobacterium; limestone; sandstone; subaerial; *Scytonema*.

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Introduction

Petalonema Berkeley ex Correns, 1898 is a small cyanobacterial genus with only 9 recognized species worldwide (Guiry and Guiry 2018). It is a terrestrial, aerophytic species that typically grows on calcareous substrates, such as dripping limestone rocks in temperate regions, particularly in the northern hemisphere, but it can also be aquatic as it has been observed in calcareous lakes in North East Europe (Kosinskaja 1926, Skuja 1929, Kukk et al. 2001) and in limestone springs (Gesierich and Kofler 2010).

Intensive literature searches revealed that the typus generis, *Petalonema alatum* (Borzi ex Bornet & Flahault) Correns, has been recorded from North America, South America, Europe, Asia and 2 islands, but no records were found for its presence in Africa or Australia (Fig.

1). A complete list of countries in which this species is recorded, is presented in Table 1.

The taxonomy of cyanobacteria has been experiencing significant changes over time, and that of *P. alatum* is no exception. *P. alatum* was first described and illustrated in 1825 under the name *Oscillatoria alata* by Captain Dugald Carmichael, a Scottish botanist, and illustrations were published in the book “Scottish cryptogamic flora” edited by Robert K. Greville in 1826 (Greville 1826). According to Carmichael the specific characters were as follows: “stratum reddish-brown, filaments brown, minute, broadly winged, wings whitish, becoming yellow towards the filament” (Greville 1826). In 1833, Miles Joseph Berkeley decided that it did not belong in the genus *Oscillatoria* because of the broad mucilage

Table 1. Distribution of *Petalonema alatum*, including references for findings.

Continent/Islands	Countries	References
North America	Canada	Poulin et al. 1995
	USA	Prescott 1962, Vinyard 1966, Wehr et al. 2015
	Panama	Drouet 1937
Caribbean	Jamaica	Drouet 1942
South America	Brazil	Sant’Anna et al. 2011
Europe	Austria	Kann 1978, Gesierich and Kofler 2010
	Britain	Berkeley 1833
	Bulgaria	Uzunov et al. 2008
	Croatia	Golubić et al. 2008
	Estonia	Skuja 1929
	Finland	Kukk et al. 2001
	France	Freytet et al. 2001
	Germany	Lemmermann 1910
	Ireland	Adams 1909
	Italy	Rizzi Longo et al. 1980
	Luxembourg	Hoffmann 1986
	Norway	Patova et al. 2015
	Poland	Starmach 1975
	Russia (European part)	Tsinzerling 1929
	Slovakia	Uher 2010
	Slovenia	Golubić 2010
	Spain	Álvarez Cobelas 1988
	Sweden	Artfakta ArtDatabanken 2018
	Switzerland	Jaag 1945, Jaki et al. 2008
	Ukraine	Kondratyeva 1968
Asia	China	Jao 1944, Hu and Wei 2006
	Phillipines	Umezaki and Modelo 1987
	India	Gupta 2012
	Japan	Nakano 1971
	Iraq	Maulood et al. 2013
	Israel	Rayss 1944, Vinogradova et al. 2000
	Azerbaijan	Mukhtarova and Jafarova 2012
	Tajikistan	Barinova et al. 2016, Barinova and Niyatbekov 2018
Oceania	New Caledonia	Couté et al. 1999

sheath surrounding the trichome, and therefore transferred it to a new genus, *Petalonema* (Berkeley 1833). In 1879, Antonino Borzi united *P. alatum*, together with *Scytonema densum*, under a new name *Scytonema alatum* (Borzi 1879). Although the morphology of *Petalonema* resembles that of *Scytonema*, Komárek and Anagnostidis (1989) stated that *P. alatum* is more closely affiliated with *Tolypothrix* (family Microchaetaceae) than *Scytonema* (family Scytonemataceae). Taton et al. (2006) and Uher (2010) agree with this statement. It is clear that the taxonomic placement of *Petalonema* has been a matter of continuous debate, especially concerning its relationship to the Families Scytonemataceae and Microchaetaceae. Taton et al. (2006) sequenced the 16S and 23S rRNA gene of *Petalonema* cf. *involvens* from Antarctica and placed them in the *Nostoc* clade. In more recent 16S rRNA gene sequencing by Mares et al. (2015), it is indicated in a phylogenetic tree that *P. alatum* formed a clade distinct from *Scytonema* species and it was proposed that *Petalonema* is a separate genus within the family Scytonemataceae. An overview of the development of main taxonomic features of *P. alatum* throughout history is given in Uher (2010).

The aim of this paper is to report the first occurrence of *P. alatum* in Africa, and to provide a distribution map, digital images, and taxonomic notes on this species found in Africa.

Methods

Samples were collected at 2 sites near the town of Clarens, in the Free State Province of South Africa. During June 2008, samples were manually collected from a sandstone overhang situated in the Golden Gate Highlands National Park (Fig. 2A; coordinates: 28°30'49" S, 028°36'59" E; altitude 2011 m above sea level). In March 2018, another overhang, called the Bushman Cave (28°34'28" S, 028°26'15" E; altitude 1872 m above sea level), not located within the boundaries of the Golden Gate Highlands National Park, was sampled. The mineralogy of the soil in the area is predominantly limestone, also known as feldspathic sandstone in South Africa, and consists of 55% quartzs, 30% feldspar, and 15% rock fragments (Johnson 1991).

A very distinctive greenish-brown biofilm mat (Fig. 2B), growing against both cave walls, was scraped from

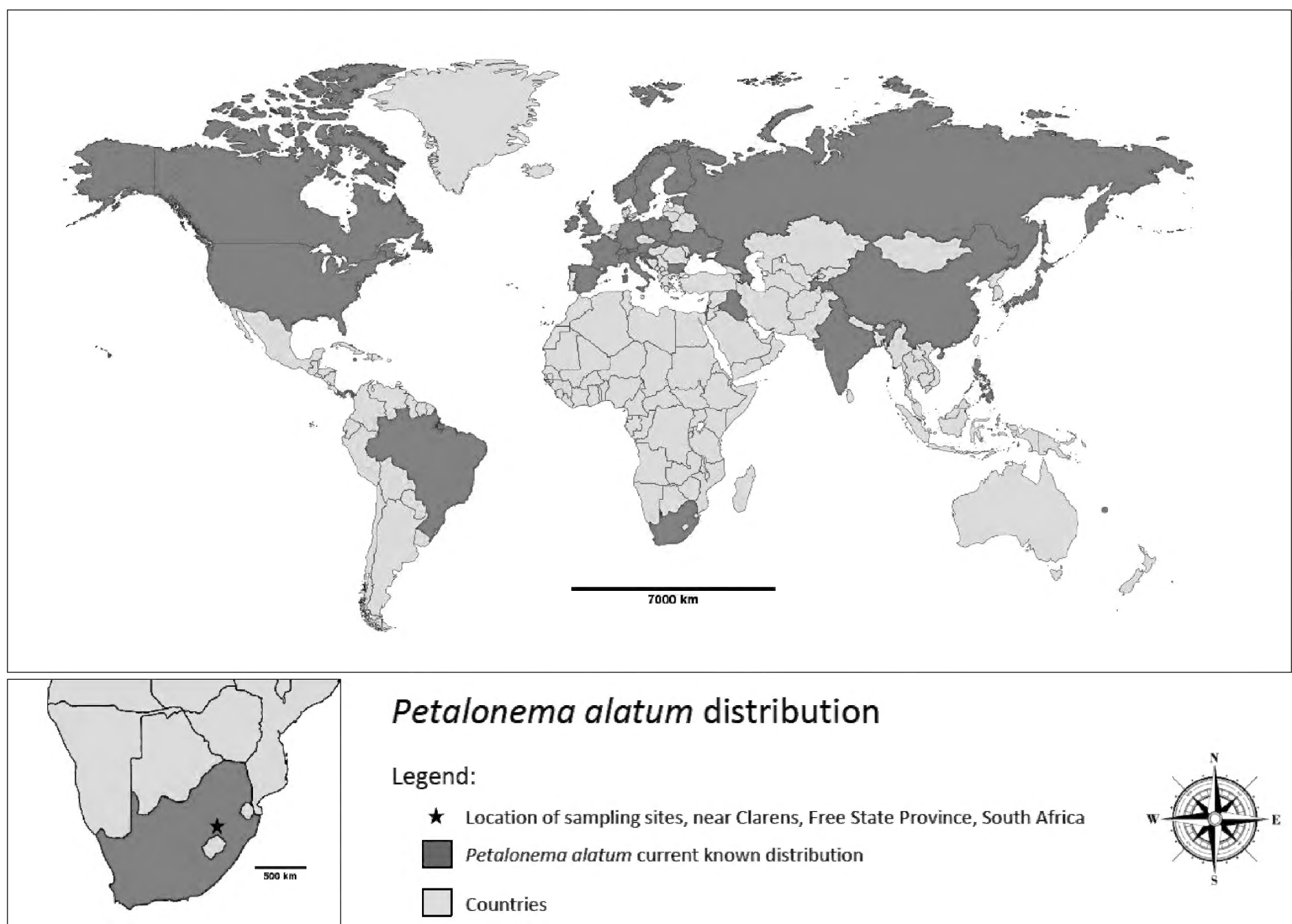


Figure 1. Current known distribution of *Petalonema alatum*.

the rock face with a sterile scalpel and transferred to sterile Whirl-Pak® sampling bags. Samples were refrigerated and transported to the Potchefstroom Campus of the North-West University. Half of each sample was transferred to a liquid GBG-11 growth medium (Krüger 1978) and agar plates (1%). Samples were incubated in a growth chamber with a light intensity of $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a temperature of 21°C . The rest of the sample was used for immediate identification with a Nikon 80i light microscope equipped with a Nikon DS-Fi1 5MP digital camera and eyepiece graticule. Light micrographs were taken of the filaments, as well as specialized structures used for species identification. Literature sources consulted for identification included John et al. (2002), Hindák (2008), Uher (2010) and Wehr et al. (2015).

The map was produced with MapChart.net, available from <https://mapchart.net/world.html>.

Results

Petalonema alatum (Borzi ex Bornet & Flahault)

Correns 1889: 321, pl. 14, figs 4–21.

Basionym. *Oscillatoria alata* Carmichael in Greville 1826: 222, figs 1–6.

Synonyms.

Petalonema alatum—(Carmichael in Greville) Berkeley 1833: 23–24, pl. 7, fig. 2a–d.

Scytonema alatum—(Greville) Borzi 1879: 373.

Scytonema alatum—Borzi ex Bornet & Flahault 1886: 89 (key), 110, fixed by Art 13(e), International Codex of Botanical Nomenclature.

Petalonema alatum—Berkeley ex Correns 1889: 321, pl. 15: figs 4–21.

Petalonema alatum—Berkeley ex Kirchner 1900: 79, fig. 57c.

New records. South Africa: Free State Province: 2 localities near Clarens:

- $28^\circ30'49''$ S, $028^\circ36'59''$ E; 2011 m above sea level, Jonathan Taylor, 12 June 2008.
- $28^\circ34'28''$ S, $028^\circ26'15''$ E; 1872 m above sea level, Louis Maree, 09 March 2018.

Sampled from the shaded rock faces of cave overhangs (Fig. 2A). Voucher specimens of *P. alatum*, sampled during 2008, were deposited in the AP Goosens Herbarium (PUC), North-West University, Potchefstroom, South Africa, accession number (PUC0014828).

Identification. Macroscopically visible mucilaginous, greenish-brown biofilms (Fig. 2B), in the form of thick mats growing in shaded parts of moist limestone rocks against the cave overhangs, were investigated and the cells corresponded to those sampled and described by Uher (2010). The base of the biofilms penetrated into the limestone substrate.

Microscopic investigations revealed the unmistakable morphology of *P. alatum* (Fig. 2C–E), characterized by blue-green trichomes surrounded by enormously wide lamellated sheaths as described by Mares et al. (2015). Our specimen's appearance also corresponded with the color photographs of *P. alatum* featuring on the front and back covers of Hindák's Color Atlas of Cyanophytes

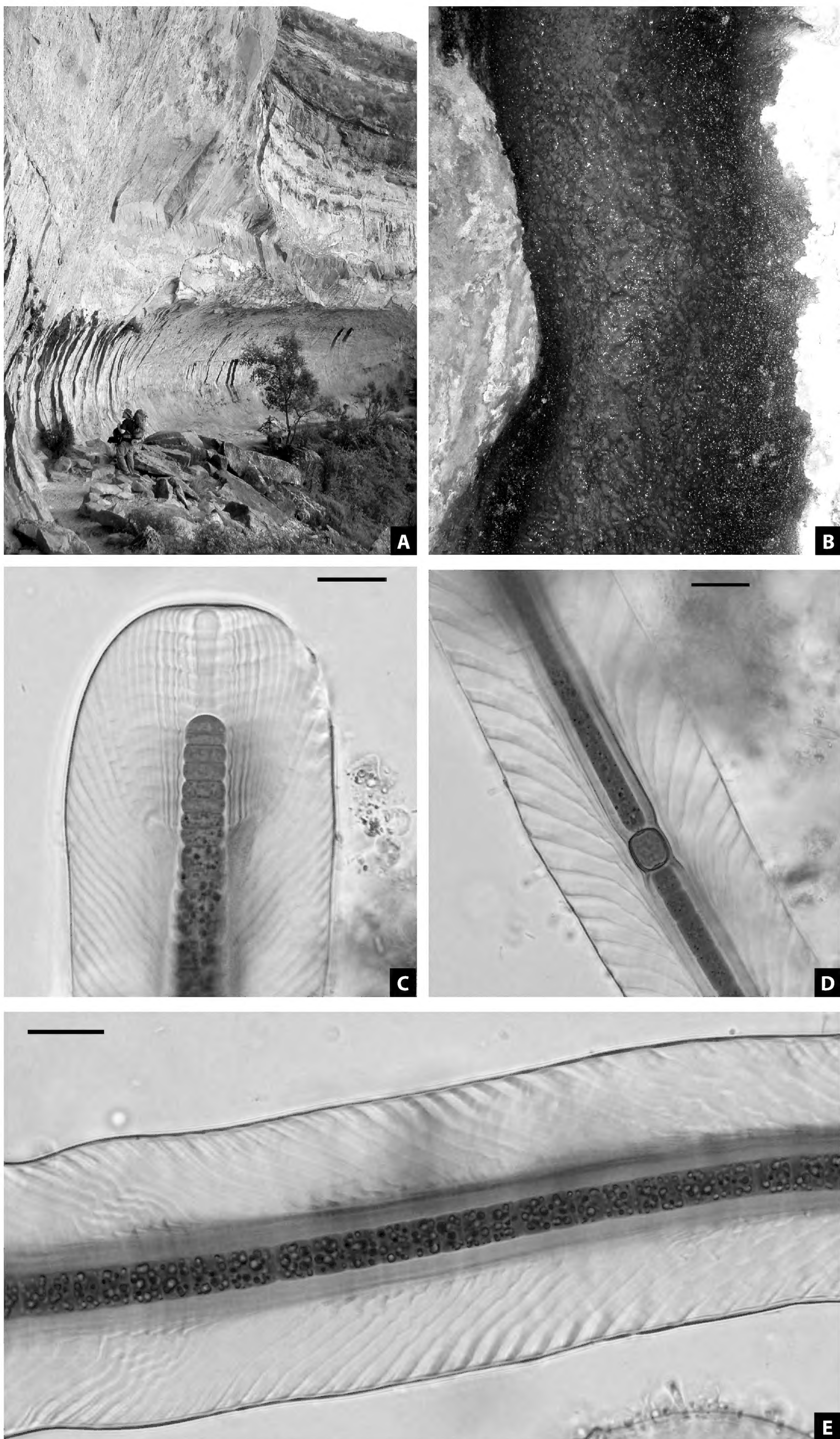


Figure 2. **A.** Sampling site in the Golden Gate Highlands National Park near Clarens. **B.** Close-up photograph of biofilm against cave wall from which samples were taken. **C.** Apical end of *P. alatum* filament. **D.** Light brown heterocyte situated in an intercalary position in the trichome. **E.** Storage products inside the cells (dark granules). Scale bars = 20 μ m.

(Hindák 2008). Trichomes were mostly straight with a width of 60–70 µm. False branching was not observed. Trichomes were constricted at the cross walls. Apical cells were rounded or globular. Cells were about 15 µm wide, 8.5 µm long and barrel-shaped. Sheaths were very thick with noticeable funnel-shaped sections tucked into one another forming divergent layers characteristic to this species (John et al. 2002, Komárek 2013). The sheaths in our samples were mostly transparent and colorless (Fig. 2). A few sheaths with yellowish tints in closer proximity to the trichomes were observed. The sheaths were always arranged parallel to the trichomes. Intercalary heterocytes appeared light brown in color and were 14.5 µm long and 12 µm wide. Cells were filled with storage products such as cyanophycin starch reserves.

Discussion

Many authors such as Borzi (1879), Correns (1898), Kosinskaja (1926), Jaag (1945), John et al. (2002), Uher (2010) and Wehr et al. (2015) presented detailed descriptions, with line drawings and/or photographs, of *P. alatum*. In accordance with the literature mentioned above, the specimen in our samples was characterized by dense clusters, forming thick biofilm mats. The sizes of the filaments, cells and heterocytes, correspond and fall within ranges given in morphological descriptions of *P. alatum* (John et al. 2002, Uher 2010, Komárek 2013, Wehr et al. 2015). The structure of the sheath, cell size, heterocyte shape and position, and patterns of branching are some of the important features used for species identification (Komárek 2013). According to Wehr et al. (2015) sheaths are often colorless at a young stage and later become yellow to brown. The presence of mostly colorless to light yellowish colored sheaths can therefore be an indication that the *P. alatum* filaments sampled were quite young.

The new record of *P. alatum* from Africa is an extremely important finding, as it extends its known geographical distribution, particularly in the southern hemisphere and the African continent. Currently *P. alatum* was only recorded from the northern hemisphere, except for Brazil (Sant'Anna et al. 2011) and the small island of New Caledonia east of Australia (Couté et al. 1999). The closest distance and direction from previous records are approximately 6,600 km north to Israel (Rayss 1944, Vinogradova et al. 2000) and approximately 7,500 km west to São Paulo in Brazil (Sant'Anna et al. 2011).

The current work forms part of a floristic survey in South Africa. Continuous investigations are recommended in order to detect new records of cyanobacteria and algae in a country and continent that are yet underexplored in terms of cyanobacterial and algal biodiversity.

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identification, as well as providing us with additional geographical distribution sites of the species. Thank you to Heinrich Voigt for his assistance in sampling. Thank you to the reviewers who provided helpful comments and made valuable suggestions to improve the manuscript.

Authors' Contributions

LM sampled the specimen, studied the geographical distribution of the species and wrote part of the text. SJvV wrote the manuscript, compiled figures and tables and liaised with experts on *P. alatum*. AL identified the species, contributed to taxonomical notes and participated in geographical distribution surveys. JT sampled the specimens and took the light microscope images.

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